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Visitor
Miss CHEN, Yvonne Stanford Universiry, U.S.A., 8 July 2002–1 September 2002

Scope of Research

This laboratory was founded in 1994 with the aim of linking (bio)chemical research and clinical medicine. Thus, the scope of our research encompasses the structure, function and pathophysiological significance of various biomolecules and bioreactions in relation to human diseases, and the application of molecular techniques to clinical diagnosis and therapy. Our current interest is focused on the role of poly(ADP-ribosyl)ation in protection of genome from apoptosis-inducing stresses, and the molecular etiology of cancer and neurodegenerative disorders including Alzheimer's disease.

Research Activities (Year 2002)

Presentations

Trp-P-1 and Trp-P-2 modulate the activity of poly(ADP-ribose) synthetase in vitro., Ueda, K., Banasik, M., Stedeford, T., and Price, D. J., 41st Annual Meeting of the Society of Toxicology, Nashville, 18 - 21 March.

Allowable chemical emissions derived from dose-response curves: Solvents as a source of confounding., Banasik, M., Ueda, K., and Stedeford, T., 12th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Vienna, 12 - 16 May.

Trans-activation of E-cadherin gene by a RING-finger protein, LUN., Oyanagi, H., Gorrin-Rivas, M. J., Chu, D., Miyahara, R., Yanagihara, K., Tanaka, H., Adachi, Y., Ueda, K., and Wada, H., 8th Central European Lung Cancer Conference, Vienna, 1 - 4 September.

A role of poly(ADP-ribosyl)ation in cell death and its regulation., Tanaka, S., Takehashi, M., Iida, S., Miwa, S., Yamasaki, K., Komeda, M., Ueda, K., 75th Annual Meeting, Jpn. Biochem. Soc., Kyoto, 14 - 17 October.

Dinucleotide repeats in monoamine oxidase A gene associated with Alzheimer's disease and Lewy body variant., Takehashi, M., Tanaka, S., Masliah, E., and Ueda, K., 18th International Congress of Clinical Chemistry and Laboratory Medicine, Kyoto, 20 - 25 October.

Advanced techniques of gene diagnosis: Non-PCR amplification., Ueda, K., 7th Asian Congress of Clinical Pathology, Kaohsiung, 6 - 9 December.

Grants

Ueda K, Special Coordination Funds for Promoting Science and Technology from the Ministry of Education, Culture, Sports, Science and Technology, 1 April 1998 - 31 March 2003.

Ueda K, Studies on diagnostic characteristics of gene testing and on the systematic reviewing of data with criteria for utility evaluation, Grant-in-Aid for Scientific Research (C), 1 April 2002 - 31 March 2003.

Tanaka S, Suppression of neuronal cell death by poly(ADP-ribose) synthetase inhibitors, Japan Foundation for Applied Enzymology, 1 April 2002 - 31 March 2003.

Intercalation activating fluorescence DNA probe and its application to homogeneous quantification of a target sequence by isothermal sequence amplification in a closed vessel

We developed a completely homogeneous and isothermal method of detecting RNA sequences and demonstrated ultra-rapid and direct quantification of pathogenic gene expression with high sensitivity. The assay is based on isothermal amplification of RNA sequence in the presence of our novel DNA probe, an INAF probe, and measurement of the fluorescence intensity of the reaction mixture. Upon detection of *mecA* gene expression in methicillin-resistant *Staphylococcus aureus* (MRSA), we could quantify initial copies ranging from 10 to 10^7 copies within 10 minutes. The primer sequences were designed to bind to secondary-structure-free sites of the target RNA, which enabled a totally isothermal protocol to quantify mRNA specifically in a sample of existing genomic DNA. When we applied this method to quantifying the expression of marker genes of *Vibrio parahaemolyticus* and *Mycobactetrium bovis*, BCG strain, the results correlated well with the viability of each bacterium. We also demonstrated monitoring *Pab* gene expression of *M. bovis* BCG during cultivation with antibiotics. The present method can potentially realize rapid antimicrobial susceptibility testing of slowly growing organisms, such as tuberculosis.

Ref. Ishigro, T., *et al.* Anal. Biochem., in press.

A possible role of poly(ADP-ribose) synthetase in neuronal degeneration

Alzheimer's disease (AD) is the most popular neurodegenerative disorder among aged people. Aβ amylod deposition is characteristic of AD brain, but its etiological role remains to be clarified. Poly(ADP-ribose) is a polymer synthesized from NAD on proteins in the nucleus of eucaryotic cells. The synthetase (PARS) requires DNA strand termini for its activity, undergoes extensive automodification, and serves as a final target of the caspase cascade in apoptosis.

In view of a concept that neuronal degeneration is a form of apoptosis, we investigated a possible role of PARS in AD pathogenesis. We observed abundant apoptosis of neuronal cells, but rarely glial cells, after treatment with Aβ peptide or NAC (non-Aβ component of AD amyloid; a fragment of α-synuclein) (Fig.1). Immunostainings with anti-PARS and anti-poly(ADP-ribose) antibodies revealed that both the enzyme and its product were distributed in the cytoplasm rather than in the nucleus of apoptotic cells.

These results, together with a finding of ROS (reactive oxygen species) production by amyloids, suggest that PARS is automodified upon ROS-induced DNA damage and cleaved by caspase, whereby losing nuclear localization and function, which eventually leads to the cell death.

Ref. Tanaka, S., *et al.* J. Neurochem. 82(2), 305-315 (2002).

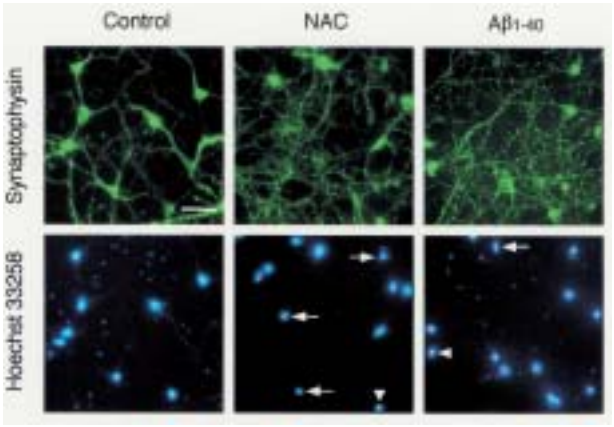

Figure 1. Induction of neuronal apoptosis by amyloid fibrils.
Cortical neurons were double-stained with anti-synaptophysin antibody and Hoechst 33258 after a 24-h incubation with 10 μM NAC or Aβ1-40 fibrils. Representative cells with condensed chromatin (arrowhead) or fragmented nucleus (arrow) are indicated. (Scale bar = 10 μm)

Dinucleotide repeats in monoamine oxidase A gene associated with Alzheimer's disease and Lewy body valiant

Monoamine oxidase (MAO) is one of the primary enzymes regulating metabolism of biogenic amines. Two distinct isoforms of the enzyme, MAOA and MAOB, have different substrate and inhibitor specificities. These enzymes are reportedly involved in the pathogenesis of Parkinson's disease (PD) through the production of oxygen radicals from catabolism of dopamine and activation of exogenous neurotoxins, such as MPTP and its analogues. The association of *MAOA* and *MAOB* gene polymorphisms with PD remains to be elucidated. Involvement of MAO in the pathogenesis of Alzheimer's disease (AD) has also been reported. However, allelic association of *MAOA* and *MAOB* genes with AD has not been rigorously analyzed.

In this study, we determined the association between (GT)n dinucleotide repeats in *MAOA* and *MAOB* gene loci and PD, pure AD, and Lewy body variant (LBV) of AD. *MAOA*-GT polymorphisms were significantly associated with pure AD and LBV. *MAOA*-GT allele 113 was excessively represented in pure AD and LBV compared with controls. Furthermore, the frequency of females homozygous for *MAOA*-GT allele 113 was higher in pure AD or LBV than controls by 2.8-fold. In contrast, there was no association between *MAOA*-GT or *MAOB*-GT polymorphisms and PD. These results suggest that polymorphisms within the *MAOA* gene may have implication in AD pathology shared by pure AD and LBV.

Ref. Takehashi, M., *et al.* Neurosci.Lett. 327(2), 79-82 (2002).